STUDIES IN EXPERIMENTAL ATHEROSCLEROSIS.

A Preliminary Report.*

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Plates 7 to 10.

In the present communication the protocols of the experiments will not be reproduced in detail, nor will the extensive literature that has gathered about the problems of atherosclerosis be discussed; and lastly, definite conclusions as to the result of the experiments will not be warranted. It is reserved for a future communication, when experiments still under way will have been concluded, and more definite knowledge as to the histological and chemical results may have been obtained, to report more in detail and with a critical appreciation of the literature.

All the investigations here reported have been done on dogs. The spontaneous occurrence of atherosclerosis in dogs, its histological structure, and the question of its greater or less analogy to human atherosclerosis are problems that have not received the attention that they deserve. In the text-books on animal pathology, little is to be found on the subject. Until recently the general opinion appears to have been that dogs were not disposed to atherosclerosis, and that if it occurred at all it was exceptional. Latterly it has been discovered that dogs are indeed subject to spontaneous atherosclerosis. The process is said to be limited, however, to the large vessels. The early stages appear as round or more oval or lengthwise patches of a whitish or pale yellowish and pinkish color, and are somewhat elevated above the surface of the intima. These sclerotic areas, it is said, are met with principally in localizations where the blood is apt to impinge with greater force against the wall of the vessel; for example, places where the vessel takes a sudden

* Received for publication, May 4, 1914.

turn in a new direction, or where other vessels branch off from the main trunk. The later stage of the atherosclerotic process is described in text-books as showing atrophy of intima and media, extensive fatty degeneration and calcification.[1] Della Vida[2] has seen spontaneous atherosclerosis in a large percentage of old dogs.[3]

Notwithstanding, however, that up to the present our knowledge of atherosclerotic processes in dogs is scant and insufficient, there seems to be no reasonable doubt that a process in many respects analogous to human atherosclerosis does occur. Granting this, for many years the writer has believed, and quite independently of experiments on dogs published in recent years, that if the possibility existed of producing experimentally a process that resembled in any way human atherosclerosis, one should be able to produce it in dogs, and not alone in rabbits. As the dog is omnivorous, it might be assumed, although it does not necessarily follow, that the metabolic conditions would approach more nearly those of human beings than is the case in the herbivorous rabbit. It is also an advantage that the disturbing median necrosis and calcification to which the aorta of the rabbit is readily disposed, is negligible in experiments on dogs. It is as yet not possible to state with certitude whether the structure of the aorta of dogs resembles the human aorta more closely than does that of the rabbit.

Much has been said and written concerning high blood pressure; various intoxications, such as lead and nicotin, and also infections with bacteria and bacterial products are important factors in the etiology of atherosclerosis. It was proposed to investigate some of these questions experimentally.

The first series of experiments was done during the greater part of 1908 and the first half of 1909.[4]

In this entire series the material to be tested was applied parenter-

[1] Hutyra, F., and Marek, J., Specielle Pathologie und Therapie der Haustiere, 3d edition, Jena, 1910, ii, 1100. Lyding, H., Zur Kenntnis der Arteriosklerose bei Haustieren, *Ztschr. f. Thiermed.*, 1907, xi, 359.

[2] Della Vida, M. L., Ueber experimentelle Arteriosklerose, *Deutsch. med. Wchnschr.*, 1913, xxxix, 2200.

[3] Celli, A., Manuale dell' igienista, 4th edition, Turin, 1912, ii, pt. 2, 1148.

[4] The writer wishes to express his thanks to Dr. William H. Park, through whose courtesy and interest it was possible to carry on this work in the laboratories of the New York Department of Health.

ally. The injections were supposed to be made into the jugular vein, with all proper regard to asepsis. It is not always easy to get the needle into the jugular vein, and it probably happened very often that the injection became subcutaneous instead of intravenous.

LEAD.

Former experience with rabbits has shown that the ordinary soluble lead salts were unsuited for hypodermic application that was to extend over any considerable length of time. The local reactions in every case were such as soon to render the injections impossible. It was found, however, that the triethylacetate of lead, as employed by Harnack[5] in his experiments, served our purpose admirably. Dr. Robert Emerson, then of Boston, prepared the salt in perfect purity. There was little or no local reaction, and the injections could thus be carried on *ad libitum*.

It should be mentioned that the blood pressure of our dogs was not tested, but that Harnack, contrary to the generally accepted opinion, found that in his animals the lead injections caused the blood pressure to rise. Two dogs were tested.

Dog 1.—The injections were commenced on Apr. 3, 1908. The animal was found dead in his cage on May 26, 1908. The symptoms corresponded closely to those described by Harnack. The temperature ranged from 101° to 104 3/5° F. There was considerable excitement, diarrhea, towards the end of the experiment bloody stools, and paralysis of hind legs. During the 55 days of the experiment the dog received injections amounting to 290 minims of a 1 per cent. solution.

Dog 2.—From June 1, 1908, to Aug. 18, 1908, when the dog was found dead. The symptoms were the same as in the first dog. The total amount injected was 590 minims. In both dogs no lesion could be found in the aorta or any other large vessel.[6]

NICOTIN.

Dog 1.—The experiment lasted from May 4 to Dec. 6, 1908, a period of about 7 months. The dog was found dead in the cage. 15 minims of a 1 per cent. solution of nicotin were injected at varying intervals, according to the condition of the dog, mostly every 2 or 3 days. There were never any convulsions. No lesions were present in the arteries.

[5] Harnack, E., Über die Wirkung des Bleis auf den thierischen Organismus, *Arch. f. exper. Path. u. Pharmakol.*, 1878, ix, 152.

[6] Harnack also failed to find any lesion in the vascular system of his dogs.

DIPHTHERIA TOXIN.[7]

Dog 1.—From May 12 to July 18, 1908,—about 67 days. The solution used was 1 : 100. Commencing with a single drop, the quantity injected was gradually increased until 1 c.c. was injected, after which the dog was found dead in the cage. No sclerotic lesions were present in any of the vessels.

Dog 2.—From Apr. 4 to Apr. 26, 1908. Death after 22 days. Temperature up to 104 1/5° F. One drop of the toxin was injected; twice only 2 drops. The result as to the arteries was negative.

Dog 3.—From Mar. 17 to Apr. 9, 1909. The solution used was 1 : 150. 1 to 10 drops were injected. Death in about 3 weeks. Temperature up to 104 1/4° F. Negative result.

EXPERIMENTS WITH BACTERIA.[8]

For the success of these experiments it was necessary to keep the animals alive as long as possible, but in a condition of mild sepsis. For this reason cultures of but moderate virulence were employed.

STAPHYLOCOCCUS AUREUS.

Dog 1.—Experiments commenced Mar. 10, 1908, and the dog was killed on June 13, 1908, after about 3 months. The injections, commencing with a single loop, suspended in normal saline, were gradually increased to the injection of an entire agar slant. The temperatures ranged from 100° to 104 2/5° F., showing the characteristic septic temperature curve. Result as to blood vessels negative.

Dog 2.—Oct. 30, 1908, to Mar. 15, 1909. Animal found dead after about 4 1/2 months. In this case the cultures were not so virulent as in the former experiment, and the sepsis was therefore not so marked, though nearly always an entire slant was injected. Temperature from 101° to 103 4/5° F. No arterial lesion.

Saltykow reported some time ago that he had obtained positive results from the injection of staphylococcus in rabbits. In his most recent publication,[9] however, he is inclined to attribute the positive result not to the injections of staphylococcus, but to the milk with which the rabbits were fed.

STREPTOCOCCUS PYOGENES ALBUS.

Dog 1.—From Oct. 30, 1908, to death, a little over 5 months, on Apr. 9, 1909. Cultures were not very virulent. Whole agar slants were used. Temperature varied in the neighborhood of 103° F. Result as to arteries negative.

[7] The diphtheria toxin was procured from the laboratories of the New York Department of Health.

[8] The cultures were obtained from the Board of Health laboratories, and were of various strains. I am also indebted to Dr. Alfred F. Hess, who controlled the purity of the cultures.

[9] Saltykow, S., Experimentelle Atherosklerose, *Beitr. z. path. Anat. u. z. allg. Path.*, 1914, lvii, 415.

COLON BACILLUS.

Dog 1.—From Mar. 10, 1908, to Oct. 15, 1908. Animal killed after about 7 months. Temperature ranged between 99 3/5° and 103 2/5° F. Animal gained weight during the experiment. Arteries normal.

Dog 2.—Oct. 30, 1908. Found dead after 5¾ months, on Apr. 17, 1909. The animal had developed a number of abscesses. Temperature ranged from 102° to 104° F. Arteries normal.

TYPHOID BACILLUS.

Dog 1.—Oct. 30, 1908. Killed after about 6 months, on Apr. 30, 1909. Temperature ranged from 101 1/2° to 103 1/5° F. Profuse diarrhea and frequent bloody stools. Arteries without lesion.

I was compelled to interrupt these studies for a number of years, but was enabled to take them up again in January, 1913.[10]

It has been shown in former experiments that the injection of adrenalin alone seemed to have no effect upon the arteries of the dog.[11] It was now proposed to test the effect of the high blood pressure caused by the adrenalin, combined with severe bodily exertion, with special reference to the purely mechanical "*Abnutzungstheorie*" that had at one time been generally and favorably received. For this purpose, a treadmill was constructed, so that two dogs could run on it simultaneously and at the same speed, and could not stand still during the period of exercise; they were, moreover, so harnessed that they could not lie down. One dog was injected with gradually increasing doses of adrenalin 1 to 1,000, usually every other day, sometimes several days in succession. It was intended to inject the adrenalin into the jugular vein, but the injections were probably mostly subcutaneous.[12]

The second dog served as a control; it received no injection, but worked steadily together with the other dog in the treadmill. The

[10] I wish to express here my grateful obligation to Professor Frederic S. Lee, Director of the Physiological Department of the College of Physicians and Surgeons of Columbia University, for placing the conveniences of his laboratory at my disposal.

[11] Otto, C., Über Arteriosklerose bei Tieren und ihr Verhältnis zur menschlichen Arteriosklerose, *Virchows Arch. f. path. Anat.*, 1911, cciii, 352. Otto finds that dogs are much less sensitive to adrenalin than rabbits, but claims some positive results in the dogs' aortas after the injection of astoundingly large quantities of adrenalin.

[12] I am indebted to Dr. B. S. Oppenheimer for carrying out the injections and for performing the autopsies on both dogs.

experiment was started on January 20, 1913. Running in the treadmill began with twenty-five minutes in the morning and thirty minutes in the afternoon. Occasionally for a day or two there was no running, but on the whole the work went on fairly steadily, and was increased gradually so that at the end of the experiment, on June 2, 1913, when both dogs were killed, they ran at top speed for almost four hours in the morning and about three hours in the afternoon. The adrenalin injections began with ten minims, and the dose was gradually increased so that at the end of about four months the dog received eighty minims at a single injection, the total amount injected in sixty injections during 130 days being 1.676 grams. These doses are minute compared to the huge quantities injected by Otto,[13] who injected 49.5 grams of pure adrenalin in the course of five months in seventy-eight injections. The initial weight of the adrenalin dog was 11,870 grams; he gradually gained in weight, so that on April 4 he reached his highest weight of 15,300 grams; from then on he gradually lost again, so that at death he had again come down to about his original weight. The control dog weighed 10,220 grams, on March 16 had gained about 2 kilos, and at death weighed about 8,780 grams. A very careful study of the blood vessels after death failed to show any arterial lesion in either dog.

It was soon noticed that, while the adrenalin dog was active and happy, and was practically untiring in running the treadmill, and always at top speed, the control dog was very soon tired, dispirited, and at the end of his forced run completely played out. It is worth noting, also, that the adrenalin dog developed no glycosuria. These facts correspond with Cannon's recently expressed views.

The uniformly negative result of all these experiments with reference to the arteries was calculated to impress one very strongly with the conviction that mere mechanical conditions, as well as intoxications and bacterial infections of various kinds, were not, at least in the dog, determining factors, and that parenteral methods were not likely to lead to any results. In the meantime, moreover, the researches of Aschoff, Anitschkow, Chalatow, Ignatowsky, Saltykow, Steinbiss, and others had been published, from which it appeared that lesions resembling human atherosclerosis very closely

[13] Otto, C., *loc. cit.*

could be produced principally in rabbits, by feeding these animals on proteins and lipoids, especially cholesterin. Following along these lines, and on the theory that atherosclerosis was probably caused by chemical rather than by mere mechanical influences, another set of experiments was undertaken to test the possible effects of various kinds of chemical substances added to the food of the animals.

From the fact that chondroitin sulphuric acid can be obtained in comparatively large quantities from the arteries, and especially the aorta, it was thought that adding this substance to the ordinary food of dogs might possibly give some results.[14]

The dog, a healthy male fox-terrier, was fed in the usual way with bread and meat. On Oct. 24, 1913, a capsule containing 0.5 gm. of the soda salt of chondroitin sulphuric acid was added to his daily meal until Nov. 25, 1913. No capsules were given until Dec. 6. After that 1 gm. of the salt was given daily until Dec. 30. The dog became ill with an abscess below the right ear, from which he recovered, and fed well most of the time. The capsules did not seem to affect him. The animal died on Jan. 18, 1914, nearly 3 months after the beginning of the experiment. His weight varied according to his general condition, decreasing when the animal was ill, increasing so that at one time he gained 1 1/4 kilos, and at his death weighing about the same as when the experiment started. The total quantity of chondroitin sulphuric acid salt consumed was 47 gm. The cholesterin content of the blood appeared to be a little below the normal, being 0.001, while the normal for dogs is about 0.00137.[15] The autopsy showed a normal aorta and large vessels.

It was now proposed to feed dogs on bread and cottonseed oil, either without any, or with only a very small quantity of protein in the food. It was assumed at first that the animals could consume about 200 cubic centimeters of oil daily, but it was soon found that about 50 cubic centimeters was all that they could manage and keep in fair condition. The low protein diet was probably not necessary.

Dog 1.—Experiment commenced Nov. 8, 1913. The oil was rapidly increased to 200 c.c. per day. For the first few days the dog ate the food. After that he would eat only one third of his daily meal, and at times he would not touch the food at all. He was killed in a fight with another dog on Dec. 6, having lost about a kilo in weight.

The cholesterin content of the blood was not determined. Autopsy showed normal vessels.

[14] I am indebted to Dr. P. A. Levene of The Rockefeller Institute for Medical Research for placing a great quantity of the chemically pure soda salt of chondroitin sulphuric acid at my disposal.

[15] Dr. Edwin Henes, Jr. (Untersuchungen über den Cholesteringehalt des menschlichen Blutes bei inneren Erkrankungen, *Deutsch. Arch. f. klin. Med.*, 1913, cxi, 122) kindly made the cholesterin determinations for me.

Dog 2.—Experiment commenced Dec. 11, 1913. Bread, 2 oz. of meat, and 150 c.c. of cottonseed oil. The dog ate all the food for 3 days, after which he sometimes took one third, one half, or sometimes the whole. On Jan. 10, 1914, the oil was reduced to 50 c.c. From then on the dog ate all that was given him, till death on Jan. 21, 1914. His weight remained about the same, till the end, when he lost about 1 kilo.

The cholesterin content was 0.00225, almost double the normal. The autopsy showed pneumonia and a duodenal ulcer. There were a number of slight fatty streaks in the aorta.

Dog 3.—Experiment commenced on Nov. 29, 1913. Bread, 2 oz. of meat, 150 c.c. of oil, which were reduced on Jan. 6 to 50 c.c. From Dec. 23 to Jan. 28 meat was taken away altogether; the dog ate bread and oil only. It was attempted to make him run in the treadmill; for a number of days he could be made to work for an hour or even more. He developed an abscess on the jaw, so that he could run only at irregular intervals. Then convulsions set in, and the running had to be stopped altogether. He was killed on Jan. 31, the experiment lasting about 2 months. The weight increased slightly at first, then decreased somewhat, but at death was about the same as at the beginning, notwithstanding attacks of vomiting and diarrhea, besides convulsions.

The cholesterin content of the blood was 0.00228, also markedly above the normal. The anatomical diagnosis and autopsy showed adherent pericardium, dilatation of heart, fatty deposits in the liver and kidneys, pulmonary emphysema, intestinal parasites, and fine fatty striæ in the aorta.

URANIUM NITRATE AND OIL.

The oil feeding was then combined with uranium nitrate.

On Nov. 19, 1913, a dog was injected subcutaneously with 1 c.c. of a solution of uranium nitrate 0.5 to 100. The injection was repeated the next day. Typical symptoms of severe uranium nephritis followed. He was fed with oil soon reduced to 50 c.c., bread, and 2 oz. of meat. On Nov. 6 meat was taken away entirely, and the dog ate well most of the time. On Jan. 11, 1914, meat was given again. The animal died on Feb. 12, 1914, nearly 3 months after the beginning of the experiment. He received altogether 6 c.c. of uranium nitrate solution in five injections. The urine at death had a specific gravity of 1.018, albumin 0.3 per cent. (Esbach), some red cells, and numerous casts, mostly hyaline. He lost steadily in weight, altogether about 2,580 gm.

The cholesterin content of the blood was low, being only 0.00013. The autopsy showed typical uranium nephritis and infarction of lung. There were no changes in the aorta or large vessels.

A second uranium oil dog died eighteen days after the beginning of the experiment, without showing any special lesions besides nephritis.

CHOLESTERIN.

On January 27, 1914, an experiment was commenced with feeding cholesterin.

Dog 1.—The dog received bread, meat, 50 c.c. of cottonseed oil, and every other day a capsule of 0.5 gm. of pure cholesterin (Merck). It was not proposed to give the large doses of cholesterin which other investigators have given to rabbits and dogs, the idea being not to go beyond normal conditions more than was absolutely necessary. The animal took his food well until about the middle of Feb.; at that time the oil was stopped, and from Feb. 25 on he took 0.5 gm. of cholesterin every day with his bread and meat. Repeated attempts to make him run on the treadmill failed, on account of his feet becoming sore after running a day or two. After about 2½ months from the beginning of the experiment, the animal died on Apr. 8, 1914. The original weight of 6,700 gm. decreased steadily, so that at death he weighed only 2,400 gm. The autopsy showed pneumonia in the lungs; there were numerous small but quite distinct fatty patches and striæ in the aorta. The total quantity of cholesterin consumed was 25 gm. The cholesterin content of the blood serum was 0.001808.

Dog 2.—Feb. 5, 1914. Bread, 2 oz. of meat, 50 c.c. of oil, and 0.5 gm. of cholesterin were given every other day. Oil was stopped after 12 days. From Feb. 25 on 0.5 gm. of cholesterin was given daily. The dog ate well from the time the oil was stopped, and was found dead on Mar. 19, 1914. His weight had decreased about 900 gm. During the 1½ months he had consumed 16 gm. of cholesterin. The cholesterin content of the blood serum was 0.00196. The autopsy showed ascites, probably due to nephritis; there was a small, cartilagenous patch in the sinus above the posterior aortic cusp, surrounded by a yellowish area of hypertrophied intima; there were besides a number of hypertrophic spots in the thoracic aorta. The bile in the gall bladder contained large quantities of doubly refracting cholesterin crystals.

HYDROCHLORIC ACID.

A casual remark of Dr. P. A. Levene suggested the simple procedure of adding dilute hydrochloric acid to the dog's food and thus producing a chronic hyperacidity.

Dog 1.—Beginning Oct. 24, 1913, at first 20, after a few days 40, then 50, and finally 60 drops of dilute muriatic acid were added to the dog's ordinary food of bread and meat. The animal took the acid without any difficulty, and fed well till the last few days before death, which occurred on Dec. 13, 1913, about 2 months after the beginning of the experiment. Though the dog took all his food and seemed perfectly well, he nevertheless lost 3 kilos. The cholesterin content of the blood was not made in this case. The autopsy showed faint yellowish streaks just above the sinuses, behind the pulmonary cusps. The aortic cusps were normal. In the abdominal aorta, about 4.5 cm. above bifurcation, several raised patches of pinkish yellowish color were seen, somewhat irregular in shape, and with the surface wrinkled, the long axis parallel to the long axis of the

vessel; there were some patches also around the orifices of efferent vessels. Transverse ridges are marked in some of these patches, especially in several that are situated in the internal iliacs. Some similar patches are also recognizable in the carotids. There was considerable edema of the left lung, but no pneumonia. The cause of death is uncertain.

Dog 2.—Beginning Dec. 22, 1913, dilute muriatic acid was added to the food, so that after 21 days the daily quantity was 60 drops, which after that was not increased. The dog was killed on Mar. 25, 1914, 3 months after the beginning of the experiment. The animal was well during the entire time, and ate greedily. He was not full grown when the experiment was started, but grew steadily, and had gained three kilos when killed. The cholesterin content of the blood serum was 0.00159, just a trifle above the normal. At autopsy the dog was found to be well nourished, with good adipose. The heart contained numerous *Filaria mitis;* the endocardium of the left ventricle was somewhat milky. No valvular lesions were present. Immediately above the aortic cusps in the sinuses of Valsalva, also near the origin of the left carotid, and especially in the abdominal aorta, and there again most pronounced in its lower part, there were greyish yellow patches slightly raised above the surface of the intima. The largest patch was situated near the origin of the innominate artery, and around this the aorta is decidedly thin. The iliac arteries showed distinct transverse striation with yellowish grey and slightly raised mottling. There was nothing abnormal in the carotid and renal arteries. Otherwise there were no gross lesions.

DISCUSSION.

In reviewing briefly the outcome of all the experiments thus far reported, one fact stands out prominently; namely, that all the injection experiments failed absolutely to yield any positive result. Neither the poisoning with nicotin or lead, nor the infections with bacteria or bacterial products, appeared to afford the slightest evidence of atherosclerotic lesion in the aorta, or in any other vessel or organ of the dogs treated. If reasoning by analogy be permitted, it may be assumed that if a bacterial infection in man, which rarely lasts longer than a few weeks, is supposed to be an important etiological factor in subsequent atherosclerosis, then bacterial infections in dogs, prolonged for many months, and finally ending in death, might reasonably be expected to cause some atherosclerotic change.

In the case of the two dogs running in the treadmill, the objection might be raised that, though the strength of the animals was taxed to the utmost, the experiment did not last long enough to cause any damage. This objection, however, loses much of its weight when one finds from the last group of experiments that positive results can be brought about in time equally short or even shorter.

This last group of experiments, though still far from concluded, has already apparently furnished some points of interest. Chondroitin sulphuric acid does not seem to be effective, and does not require further discussion. All the other experiments, with the exception of two dogs to whom dilute hydrochloric acid was given, were undertaken for the purpose of studying the now generally conceded causal relation between cholesterin and the atherosclerotic process in man, as well as in the rabbit. Pure cholesterin (Merck) was given only in two cases. The other dogs took cottonseed oil, not only because this was less expensive than cholesterin, but also because it was thought probable that this oil contained sufficient lipoid material to answer every purpose. It was not proposed to administer the colossal quantities of cholesterin which some investigators had employed, and which exceeded by far all that either man or the rabbit would ever consume under natural conditions.

In all the oil and cholesterin dogs, with but one exception, atherosclerotic lesions were found. It is true, however, that only the very earliest stages appeared; *i. e.,* the fine yellowish striæ, and the fatty patches, in their gross appearance closely resembling the same structures so familiar in the human aorta.

The one exception was the dog with uranium nephritis. On general principles one would have expected that an oil-fed dog with chronic uranium nephritis, would be much more likely to develop atherosclerosis than a dog with sound kidneys. This seems not to have been the case. Neither in the aorta, the renal artery, nor in the kidneys, in fact in none of the vessels or organs of the nephritic dog, could any trace of atherosclerosis be found. The cholesterin content of the blood serum in this dog was much lower than in any other dog. It will be the subject for further study to arrive at an understanding regarding these phenomena.

In the aorta of the first acid-fed dog, as described above, there were found not only the fine, fatty striæ in the thoracic aorta, but quite large raised sclerotic patches in the lower portion of the abdominal aorta and in the external iliacs. These patches closely resembled those atherosclerotic areas in human beings that have not yet become ulcerated or calcified. This dog, however, was about four years old. The obvious objection was therefore close at hand

that the atherosclerosis in his case might have been spontaneous and entirely independent of the acid feeding. A young and growing dog was therefore subjected to the same treatment, and for a somewhat longer time, with the result that atherosclerotic changes similar to those in the first dog were now manifest over a considerably greater area of the aorta, both thoracic and abdominal, but more pronounced in the abdominal aorta. Though only two dogs have thus far been fed with hydrochloric acid, the possibility can not be denied, especially in view of the numerous negative results with other methods, that these positive results are not mere coincidences, but are probably due to the hydrochloric acid. Further investigations are necessary, and are under way.[16]

While writing this communication, the work of Oswald Loeb[17] has come to my notice. He describes results similar to mine in two dogs which he fed with large doses of lactic acid and kept on an exceedingly low protein diet. The detailed reports of his experiments, and especially the microscopic findings, have not yet been published. No diminished protein diet was given to our dogs. They fed well on their usual diet of bread and meat, thus showing that the sclerotic affection of the large vessels was in all likelihood due to the acid, and that it was probably immaterial whether the dogs ate meat or not.

The histological structure of this canine atherosclerosis has not yet been completely studied in all its details, but it has been ascertained that the lesions are primarily and principally localized in the intima. The intima of the dog's aorta, however, appears to differ somewhat in its structure from that as found in man and in the rabbit. It would appear that under normal conditions the elastica interna is covered by a single layer, possibly by several, fine layers of endothelial cells. Fibrous or muscular tissue is nowhere perceptible. The very earliest beginning of the sclerotic process is marked by a proliferation of rather large, flat cells, in all probability of en-

[16] Since writing the above, I have examined a third dog. The animal, scarcely one year old, died of pneumonia after having had acid feeding only for 3 weeks. He, too, showed a number of typical yellowish patches in the arch of the aorta.

[17] Loeb, O., Ueber experimentelle Arterienveränderungen mit besonderer Berücksichtigung der Wirkung der Milchsäure auf Grund eigener Versuche, *Deutsch. med. Wchnschr.*, 1913, xxxix, 1819.

dothelial origin. Notwithstanding protracted search, and aided by the best technical methods and all sorts of stains, nothing in any way resembling the macrophages or the large amebic leucocytes described by Saltykow could be found (figure 1). Almost simultaneously with the appearance of these large cells, elastic fibers begin to be split off from the elastica interna, and the whole process, as described by Jores, takes place (figure 2). A larger or smaller elevation or excrescence is thus formed, the base of which is still the elastica interna, and for some distance there can also be recognized one or two rows of those peculiar large endothelial-like cells, at the base of the elevation, and resting immediately upon the elastica. Between the newly split off elastic fibers which form, as it were, the skeleton of the little excrescence, there now develops a more or less swollen and succulent connective tissue, and possibly also some muscular fibers. In the meanwhile, and in that portion of the media underlying the newly formed elevated patch, the elastic fibers are beginning to give way, and the tissues there are also in a swollen and succulent state. No aggregation of leucocytes, or anything else pointing to an inflammatory process, could be made out. In some text-books it is stated that calcification has been found in the spontaneous atherosclerosis of dogs, and it is perhaps not improbable that calcification would soon have followed the developments just described, had the experiments lasted sufficiently long. Further investigation is required to clear up this point. It does not appear unreasonable to presume that the calcium metabolism in dogs, as well as in other omnivorous animals, differs from that of the rabbit, in which calcification, primarily in the media, occurs early and often spontaneously.

Simultaneously with the first appearance of the proliferating cells, and only among them and not in the normal intima, minute sudanophil droplets are seen contained in the upper portions of the proliferating cells, and also lying free above and between them. Very soon they are found in the media and down to the adventitia. As the process proceeds, the droplets become larger, sometimes run together, but never have we found them of the size and number as described in the rabbit and in man. They are most numerous in those fatty striæ and elevated fatty patches that we

found in the aortas of the oil-fed, and especially of the cholesterin-fed dogs (figure 3), but they also occur with great constancy, though much smaller in size, in the acid-fed dogs. They stain readily with Sudan III and Scharlach R; but they are optically inactive, and therefore are probably not cholesterin, but some other as yet undetermined fatty or lipoid substance. It is a striking fact that, while the bile of the cholesterin-fed dogs contained innumerable doubly refracting cholesterin crystals, not a single one could be found in the arteries, the liver, and the adrenals. Chalatow[18] has recently pointed out that the deposit of the doubly refracting so called fluid crystals, most abundant in the rabbit, does not take place in all animals. It would seem from the observations on our dogs, that, though the cholesterin content of the blood serum may be high, there is nevertheless no permanent deposit of cholesterin in the organs, and it is probable that there is a rapid elimination.

It is noteworthy, also, that the acid-fed dogs showed numerous sudanophil droplets in the early stages of the sclerotic hypertrophy; but that these sudanophil bodies had almost, if not entirely disappeared by the time the fully formed sclerotic area had developed.

The livers of all the dogs, as well as the adrenals, were crowded with sudanophil bodies. It could be shown that there was no fatty degeneration, but merely an overcrowding of the cells, and even to some extent of the intercellular tissue, with an infiltration of fatty material. Doubly refracting components could never be found. The livers of the acid-fed dogs, more than those of any of the other animals, were filled with this fatty substance, and it was especially the cylindrical epithelium of the bile ducts that was most conspicuously loaded with brilliant sudanophil matter (figure 4).

The work is being continued, and definite conclusions would at this stage be premature; but perhaps it may be permitted, even now, to venture the statement that in all probability the theory which bases atherosclerosis on a purely mechanical etiology will not prove tenable. Whether mechanical factors come into play at all, and if so, to what extent, remains to be seen.

It seems almost certain, at least in our present state of knowledge,

[18] Chalatow, S. S., Über flüssige Kristalle im tierischen Organismus, deren Entstehungsbedingungen und Eigenschaften, *Frankfurt. Ztschr. f. Path.*, 1913, xiii, 189.

FIG. 1.

(Adler: Studies in Experimental Atherosclerosis.)

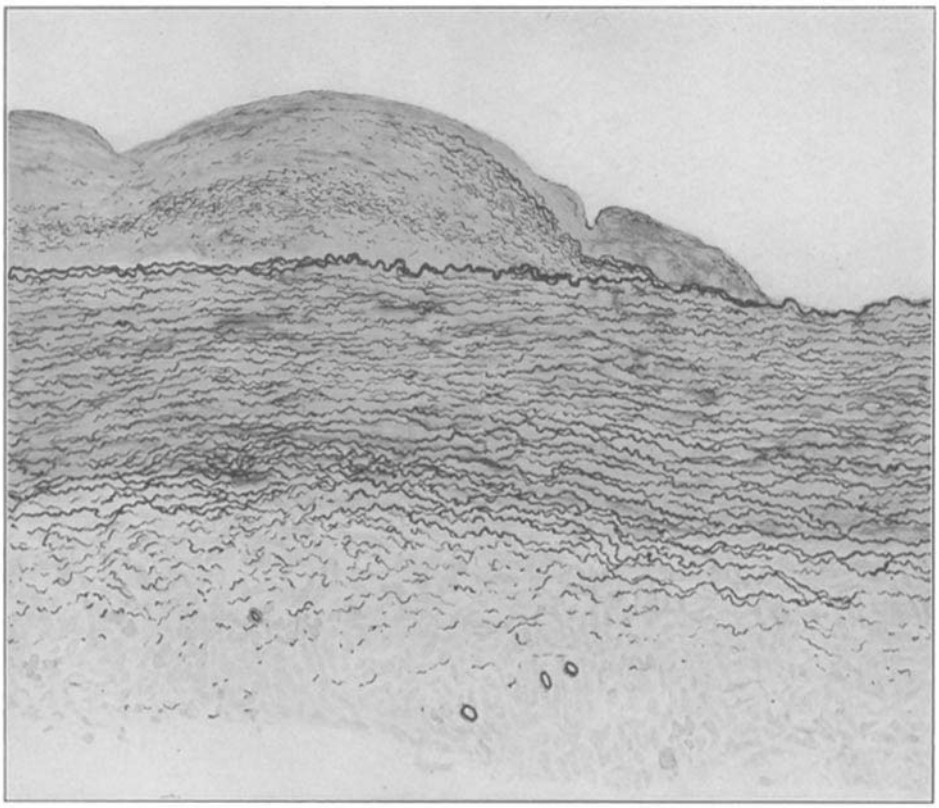

FIG. 2.

(Adler: Studies in Experimental Atherosclerosis.)

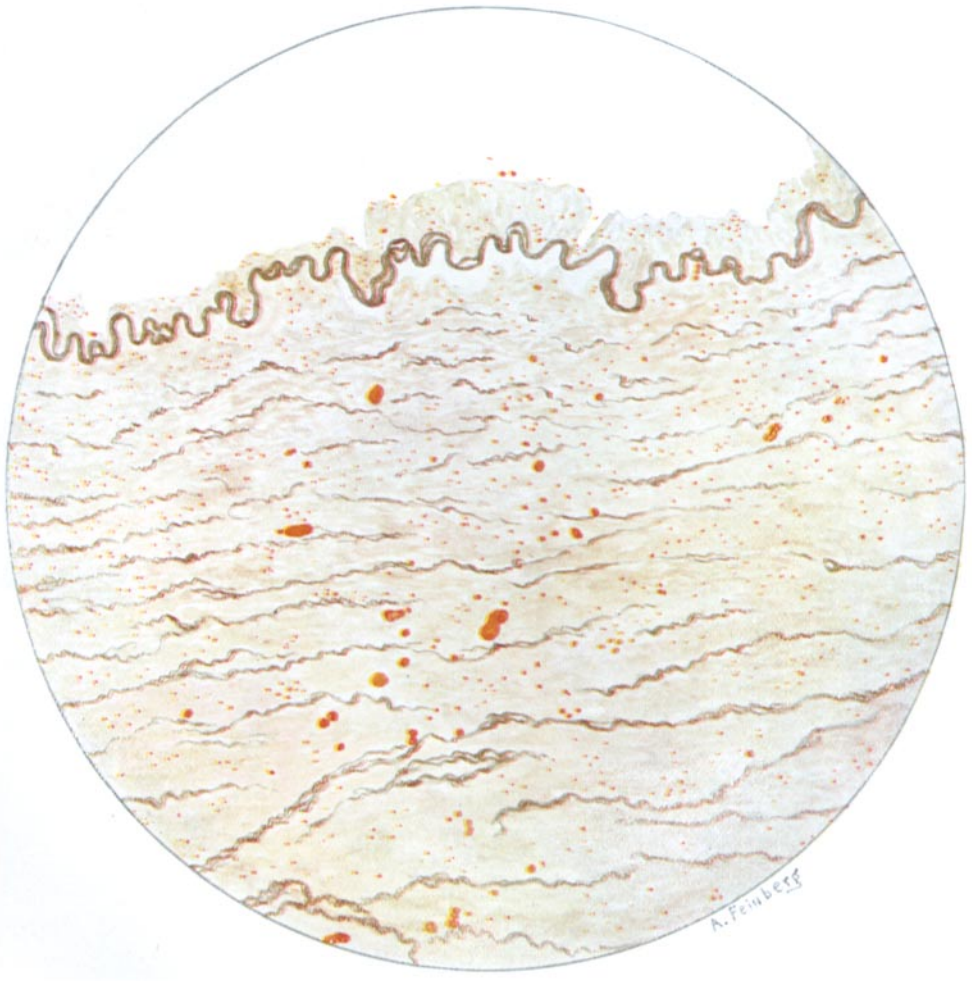

FIG. 3.

(Adler: Studies in Experimental Atherosclerosis.)

FIG. 4.

(Adler: Studies in Experimental Atherosclerosis.)

that chemical influences, subject possibly to more or less nerve control, are dominant factors in the etiology of atherosclerosis. Perhaps it may be discovered also that cholesterin and its various modifications and combinations, while undoubtedly an element of importance in atherosclerosis of the rabbit and human beings, may not be the sole predominant etiological factor. If it should turn out that so simple a procedure as adding a certain proportion of hydrochloric acid to the food of dogs is sufficient to produce lesions of the blood vessels closely analogous, if not wholly identical with human atherosclerosis, a revision of our present theories will become necessary.

EXPLANATION OF PLATES.

PLATE 7.

FIG. 1. Section through a sclerotic patch from the aorta of an acid-fed dog. Weigert-Van Gieson stain shows the proliferation of endothelial cells and the typical hypertrophy of the intima.

PLATE 8.

FIG. 2. Section through the same aorta. Weigert elastic stain shows splitting off of elastic fibers from the elastica interna.

PLATE 9.

FIG. 3. Frozen section through a yellow patch of the aorta of an oil-fed dog. Sudan III shows incipient proliferation of the endothelium and distribution of sudanophil bodies.

PLATE 10.

FIG. 4. Frozen section through the liver of an acid-fed dog. Stained with Sudan III. The liver cells and especially the cells of the bile ducts are crowded with sudanophil bodies.